

Immunologic Responses to Inhaled Cotton Dust

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Byssinosis, a respiratory disease of workers on cotton, flax, and soft hemp, is classically characterized as shortness of breath, cough, and chest tightness on Mondays or the first day of return to work after a time off. Exposure to these vegetable dusts can also result in other respiratory diseases, and the term cotton dust-induced respiratory disease (CDIRD) is introduced. Although clinically characterized for more than a century, the underlying pathogenesis of CDIRD remains obscure. An allergic pathogenesis has been proposed. This article reviews previous and current research findings supporting this mechanism and raises the possibility that, in some individuals, CDIRD may be due to pre-existing or occupationally induced mold allergy.

Current public preoccupation with occupational health hazards suggests that these are the result of a modern industrialized society. Occupational diseases were, however, first described by the Greeks and Romans. By the 4th century B.C., hazards in the mining industry had been recognized, although at that time protection of workers and prevention of disease were not concerns. Five hundred years later, Pliny the Elder described a bladder-derived mask to protect laborers against lead dust and fumes. In 1556, Georgius Agricola suggested mine ventilation, recommended use of protective masks, and described the disease now known as silicosis (1). The first generally accepted treatise on occupational hazards in the workplace was *De Morbis Artificum* by Bernardo Ramazzini. Published in 1713, this book described many occupational lung diseases, including those of farmers, bakers and millers, grain measurers, and hemp workers (2).

It is noteworthy that many of the illnesses described by Ramazzini resulted from exposure to organic dusts. Today, dusts from wood, grain (and flour), and textile fibers and/or microorganisms contaminating these dusts account for a large number of airway disorders of occupational origin (3). A wide range of occupational disease types occur, the nature of which depends on: (1) the site of maximal deposition, which in turn is dictated by physical properties of the agent; (2) the type of reaction generated, which frequently depends on the chemical properties of the inhalant; and (3) host factors, including genetic and environmental influences. Occupational lung diseases resulting from inhaled organic dusts include:

asthma, hypersensitivity pneumonitis, bronchitis, and certain "flu-like" illnesses such as those resulting from endotoxin exposure. Complaints may occur singly or as part of a "symptom complex," as seen in workers exposed to cotton, flax, or hemp dusts (4).

Ramazzini described the respiratory problems seen in textile workers: "... For a foul and poisonous dust flies out of these materials, enters the mouth, then the throat and lungs, makes the workmen cough incessantly, and by degrees brings on asthmatic troubles... one may see these men always covered with dust from the hemp, pasty-faced, coughing, asthmatic, and blear-eyed... they cannot help taking in foul particles by the mouth; these pollute the spirits and stuff up the organs of respiration..." This disease was not yet recognized in cotton workers since, prior to the Industrial Revolution, cheap and high quality cotton goods were not widely available. After the invention of the cotton gin, however, English mills greatly increased their production, and there was a concomitant increase of air pollution in the mills and a rise in respiratory illnesses among workers. By 1818, bronchitis and "pulmonary maladies" among cotton operatives had been noted (5); by 1831, the relationship between dust-induced illness and work in cardrooms had been established (6). Although the term byssinosis, for the respiratory disease affecting cotton, flax, and hemp workers, was not used until 1877 (7), its cardinal symptom was first described by Mareska and Heyman, two Belgian physicians, in 1845: "... All workers declared that the dust troubled them much less on the last days of the week than on Monday and Tuesday..." (8).

Today, by definition, byssinosis is chest tightness and/or shortness of breath occurring on Monday or the first day of work after a time off. These symptoms abate over-

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Table 1. Byssinosis severity.

Grade	Symptoms
Grade 0	No symptoms of byssinosis
Grade 1/2	Occasional chest tightness on the first day of the work week
Grade 1	Chest tightness on every first day of the working week
Grade 2	Chest tightness on the first and other days of the week
Grade 3	Originally described as Grade 2 symptoms accompanied by evidence of permanent ventilatory impairment

night and, if they recur on Tuesday, are usually milder. Schilling and co-workers (9) used this periodicity of symptoms for the original grading system of byssinosis severity (Table 1).

It is apparent, however, that exposure to dusts generated during the processing of cotton, flax, and hemp may be associated with several responses: (1) the acute reaction described above, (2) a decline in ventilatory capacity on Monday, and (3) mill fever, which is a symptom complex occurring when an individual originally enters the mill or returns after a long absence (10). In addition, there are reports of an increased prevalence of bronchitis (11,12) and chronic obstructive pulmonary disease (COPD), either progressing from the acute disease or occurring as a separate entity (13-15). These latter findings are not universal (16), and although COPD resulting from occupational exposure to cotton dust is a major public health concern, it is beyond the scope of this article. With these varied features, cotton dust-induced respiratory disease (CDIRD) may be a more appropriate term than byssinosis.

Although clinically characterized for more than a century, neither the etiologic agent nor the pathogenic mechanism(s) of CDIRD has been determined. A major problem with evaluation of the causative agent(s) or disease mechanisms is the extreme heterogeneity of cotton dust. Cotton dust is, by definition, a "dust generated into the atmosphere as a result of the processing of cotton fibers combined with naturally occurring materials such as stems, leaves, bract, and inorganic matter which may have accumulated on the cotton fibers during the growing or harvesting period" (17). In addition, cotton dust is heavily contaminated by gram-positive and gram-negative bacteria and molds (18-22) (Table 2). Aqueous extracts of cotton dust, plant parts, and microbial agents exhibit a wide variety of biologic responses including, but not limited to: activation of the alternative pathway of complement (23), chemotaxis (24), pharmacologic mediator release (25,26), smooth muscle contraction (27), and ability to induce antibody production (28,29). These varied actions are reflected in the proposed pathogenic mechanisms.

Several hypotheses, based on these biologic responses, have been put forth to explain the pathogenesis of CDIRD. These have been recently reviewed (30,31), but, briefly, can be grouped into three primary headings: (1) pharmacologic, which includes actions on tissues result-

ing from nonspecific mediator release and/or a direct action of dust components on smooth muscle; (2) microbiologic, which involves bacteria and/or their metabolic products, notably endotoxin; and (3) immunologic, including humoral antibody production. This review will center around the evidence for and against an antigen-antibody induced disease.

An immunologic pathogenesis is attractive since there is a lag time between the initial dust exposure and disease development, and not all dust-exposed individuals develop CDIRD, suggesting the involvement of genetically determined host factors, such as an allergic diathesis (i.e., atopy) or specific histocompatibility type. Although earlier evaluations of immunologic responses to cotton dust exposure often yielded equivocal results, recent studies, discussed below, provide strong evidence for one or more types of hypersensitivity in disease pathogenesis.

Early studies by Taylor and colleagues (32,33) suggested that specific precipitating "antibodies" directed against a condensed tannin extracted from cotton plant parts, were present in higher titers in sera from cardroom workers than controls. This "antigen" was subsequently identified as a polymer of 5,7,3', 4'-tetrahydroxyflavan-3,4-diol (THF). Edwards and Jones (34) subsequently demonstrated the pseudo-immune nature of this reaction. When pooled sera from patients with farmer's lung were tested against THF, 58% of the IgG, 54% of the IgM, and 15% of the IgA present in the pool bound to the "antigen." Further, the Fc fragment of an IgG molecule, which cannot bind antigen, also produced a precipitin reaction. Kutz and co-workers (35) demonstrated that precipitation was due to β -lipoprotein reacting with a tanninlike material in the cotton extract. These findings clearly indicate a nonimmune reaction, although the possibility remains that these complexes can activate complement.

When tested against cotton dust extracts, sera from some cotton textile mill workers demonstrated precipitins by double gel diffusion or counter immunoelectrophoresis. These reactions appeared to be nonspecific and did not correlate with the presence of disease (35-37). Thus, if an immune response does play a role in CDIRD, it is likely to be a Type I, IgE-mediated hypersensitivity (38), rather than one involving precipitating or agglutinating antibodies.

Table 2. Composite of microbial genera identified in cotton dust, cotton plant parts, and/or textile mill air.

Gram-negative bacteria	Gram-positive bacteria	Actinomyces	Fungi
Enterobacter	Bacillus	Thermoactinomyces	Aspergillus
Pseudomonas	Clostridium		Penicillium
Agrobacterium			Cladosporium
Actinobacter			Fusarium
Escherichia			Alternaria
Flavobacterium			Rhizopus
Klebsiella			Mucor

The acute byssinotic response is unlikely to be a form of classic "immediate onset," IgE mediated asthma, since an immediate asthmatic reaction is associated with a rapid response (10–15 min), whereas symptoms of chest tightness and/or declines in ventilatory function develop over 4–6 hr in byssinotics (39). Thus, although wheezing is not generally considered to be a symptom, byssinosis may be a form of "late onset," IgE-dependent asthma. Some byssinotic individuals may, however, be suffering from an IgE-mediated hypersensitivity to a cotton dust contaminant and be "misdiagnosed" as byssinotic.

Early studies of an allergic response mechanism of CDIRD were reviewed by Prausnitz (40), who concluded, from his own work, that there was a "distinct supersensitiveness" to cotton dust protein in operatives suffering from disease. Subsequent studies were unable to confirm these results and showed that there were no differences in the incidence of positive skin tests among advanced byssinotics, atopics, or normals (41).

It is not always clear from these early studies if immediate or late phase reactions were observed. Cayton and colleagues, however, stated that late skin reactions were seen in 90% of the individuals tested. They felt that this high response rate was due to a toxic element(s) in the dust rather than a specific antibody response. The chemical nature of these extracts and house dust extracts was then examined and it was demonstrated that both contained nearly the same amino acids, and in this respect, they resembled molds (42). Results from skin testing and provocative challenge of byssinotics with fungal extracts have been inconclusive (42–45). Although some positive reactions have been obtained, these did not correlate with the presence of disease. Studies also indicated that positive skin reactions could be elicited in normal volunteers.

The discovery of immunoglobulin E (IgE) and development of *in vitro* assays, such as the radioallergosorbent test (RAST), have revolutionized the study of allergic disorders. Such advances have contributed to multidisciplinary studies in cotton mills. Our study of oil mill workers (28) is an example. Workers ($n = 230$) were categorized into dust exposure groups: cotton linter, product (meal dust, etc.), and mixed linter and product. Individuals were skin prick-tested with a panel of common inhalant allergens, to determine their atopic status, and with cotton linter and seed extracts to evaluate specific cotton immunoreactivity. Blood was drawn for subsequent examination by RAST; a questionnaire was administered to obtain symptom history; area and personal levels of dust exposure were measured; and pre-post work shift spirometry was performed, to evaluate exposure related pulmonary function changes. For the purposes of this study, atopy was defined as the presence of two or more positive skin test reactions to the inhalant allergens.

In the linter-exposed group, atopic individuals had a greater post-shift decline in forced expiratory volume at 1 second (FEV_1) than nonatopics. Further, workers exposed to cotton linters had the highest levels of skin reac-

tivity to both cotton linter and seed extracts when compared with other dust exposed groups. *Fusarium*, a fungal extract included in the test panel, also showed this trend. In the linter-exposed workers, a correlation was also shown between decline in pulmonary function and positive skin prick test reactions to linter and/or cotton seed extracts, and *Fusarium*, *Alternaria*, and Johnson and Bermuda Grass.

Subsequently, we studied employees of cotton textile mills, working in areas up to, but not including, weaving. As a control population, workers processing synthetic textile fibers were tested. The format of this study was similar to those previously described for the oil mills. In these studies, a standard cotton dust extract (ACDE), prepared from Stonewall V-cell dust (1978), and mill specific dust extract (either cotton or synthetic fiber) were used to assess cotton reactivity.

To date, we have tested 295 individuals employed in cotton textile mills, and 101 employed in synthetic fiber factories. Results from skin testing indicated that 91 individuals reacted to one or more of the dust extracts. Of these workers, 26 reacted to ACDE alone, 9 reacted only to the mill specific dust, and 55 reacted to both. It is noteworthy that individuals working in synthetic fiber mills where no cotton products *per se* were used, also had positive skin reactions to cotton dust. This indicates the presence of a "non-cotton" allergen, common to both synthetic fiber and cotton dust extracts, or an allergen present in the general environment, such as mold or pollen (46).

Comparisons of specific ACDE reactivity with the atopic status of workers are shown in Figures 1 and 2. Of the 295 cotton workers tested, 45 (15%) were atopic and, of these, 27 (60%) were reactive to ACDE. Of the 250 nonatopic individuals tested, only 31 (12%) reacted to ACDE. Results were similar in the synthetic fiber

	Atopy		Total
	Yes	No	
ACDE Prick Test	(+)	27	31
	(-)	18	219
Total	45	250	295

FIGURE 1. Atopic status of workers employed in cotton textile mills compared with skin reactivity to aqueous cotton dust extract (ACDE). $\chi^2 = 54.70$ ($p < 0.0005$).

ACDE Prick Test	Atopy		Total
	Yes	No	
	(+)	(-)	
	11	12	23
	7	71	78
Total	18	83	101

FIGURE 2. Atopic status of workers employed in synthetic fiber mills compared with skin reactivity to aqueous cotton dust extract (ACDE). $\chi^2 = 18.31$ ($p < 0.0005$).

textile mills: 18/101 (18%) were atopic and 11 (61%) were positive to ACDE; of the nonatopic individuals tested, only 12/83 (14%) were reactive to ACDE. In both populations, there was a significant relationship between the presence of atopy and cotton dust extract immunoreac-

Table 3. Results from radioallergosorbent testing (RAST) on reactive individuals from textile mills tested in two consecutive years.^a

ID No.	Year 1	Year 2
1	2.00	2.13
2	1.60	3.08
3	2.50	3.10
4	1.50	2.50
5	2.90	5.90
6	1.60	2.89
7	1.30	2.29
8	1.50	2.34
9	1.70	2.29
10	2.20	3.25
11	1.20	2.28
12	1.20	2.56
13	4.10	8.99
14	2.20	11.54
15	1.62	3.70
16	3.20	2.68
17	1.50	2.19
18	3.30	4.78
19	1.60	2.30
20	1.20	3.42
21	0.90	2.60
22	1.50	3.83
23	2.00	2.53
24	1.30	3.20
25	2.50	8.58
26	1.70	2.16
27	1.60	2.22
28	2.10	3.46

^a Results are expressed as a ratio of test disc counts per minute (cpm)/control (human serum albumin) cpm. A ratio greater than or equal to two is considered positive.

Table 4. Results of immunologic and pulmonary function tests on volunteers exposed 6 hr in a model card room.

ID No.	Post exposure change FEV ₁ %	Post exposure symptoms	Total IgE, IU/mL	RAST ratio ACDE	Atopic history
1	-12	Yes	130	1.9	Yes
2	-1	Yes	323	2.4	Yes
3	-1	Yes	373	2.1	Yes
4	-13	Yes	39	2.0	No
5	-5	Yes	865	2.8	No
6	-5	No	420	3.0	No
7	-1	Yes	18	3.0	No
8	-12	Yes	903	3.5	No
9	-5	No	9	1.6	No
10	-3	Yes	152	3.4	Yes
11	+1	Yes	14	1.9	No

tivity. These data complement the findings from studies of workers employed in oil mills.

It is not clear if workers enter the textile mill with pre-existing allergy to a common dust component or develop sensitization concomitantly with mill employment. Results from RAST, over two consecutive years, of workers employed in the textile mills suggested that continued exposure to cotton dust can increase both the incidence and level of specific IgE antibodies (Table 3). On the other hand, results from our immunologic studies of volunteers exposed in the model cardroom at Clemson, SC, indicated that specific IgE antibodies against a dust contaminant can be present without prior occupational exposure (37). This suggests that workers may enter the mill with specific "anti-cotton dust" antibodies.

In the Clemson study, a total of 32 individuals, most without prior exposure to cotton dust, were exposed for 6 hr to 1 mg/m³ of Standard Stoneville cotton. Pulmonary function testing was performed pre/post exposure (results were kindly supplied by Dr. Brian Boehlecke of NIOSH). Blood samples for additional testing were collected on the first 11 individuals who agreed to participate in our immunologic studies. Levels of total serum IgG, IgA, IgM, IgD, and IgE; specific IgE anti-ACDE antibodies; and specific ACDE-precipitating antibodies were determined. Total immunoglobulin levels were all within normal limits and no precipitating antibodies to crude dust extract were detectable. Eight of the eleven subjects tested had a positive RAST value and six of

Table 5. Relationship of atopic status of workers in industries where occupational asthma occurs and the suspected pathogenic mechanism of disease.

Factory	No. atopics/ no. tested (%) ^a	Pathogenesis
Coffee processing	46/358 (12.8)	IgE mediated
Textile manufacture	62/394 (15.7)	IgE component
Seed oil manufacture	56/349 (16.0)	IgE component
Toluene diisocyanate (TDI) manufacture	52/186 (28.0)	Pharmacologic (?)
TDI use	107/325 (32.9)	Pharmacologic (?)

^a Atopics identified by the presence of two or more positive skin prick tests to a panel of 10 common inhalant allergens.

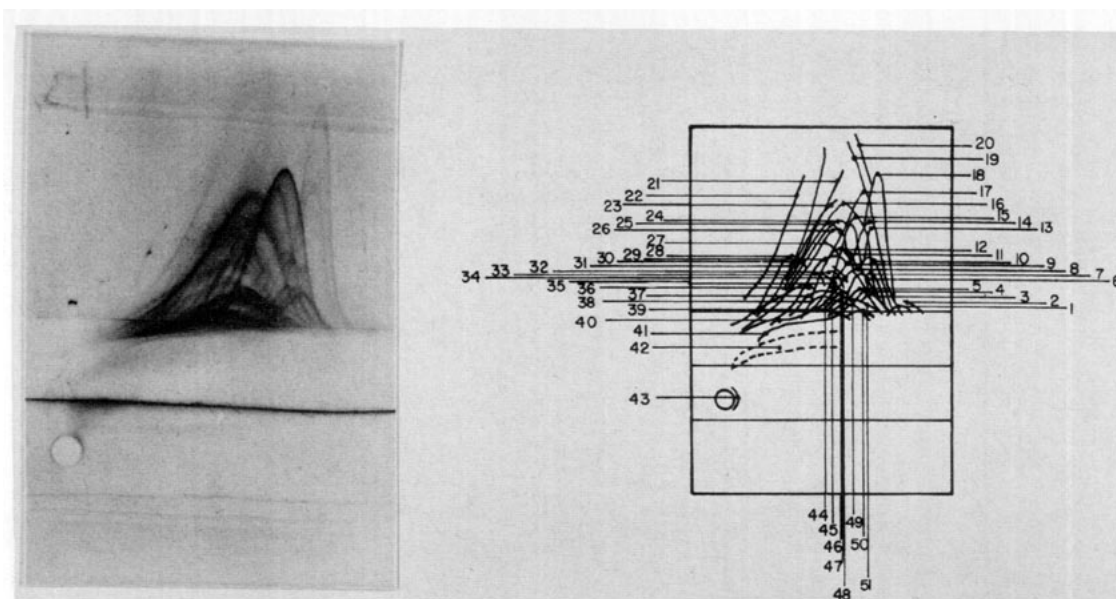


FIGURE 3. Crossed immunoelectrophoresis (CIE) pattern of aqueous cotton dust extract (ACDE) and hyperimmune rabbit anti-ACDE antisera: (a.) Stained plate b. line drawing with peaks arbitrarily numbered. From O'Neil et al. (29). Reproduced with permission of S. Karger AG, Basel.

these had elevated total IgE levels. Increased IgE values (greater than 300 IU/mL) are suggestive of atopy. Two of these volunteers had chest tightness, three others had respiratory symptoms, and one reported no symptoms. The individual with the highest RAST score (3.5) and symptoms of chest tightness had a 12% decline in FEV₁. These data are summarized in Table 4.

These findings suggest that there is an IgE-mediated component in the pathogenesis of CDIRD. Further indirect support for this hypothesis comes from determining the prevalence of atopy in individuals working in industries where occupational lung disease occurs. Workers employed in coffee processing, cotton seed crushing (oil mills), and textile manufacturing, where occupational lung disease is known or suspected to occur via an IgE-mediated mechanism, had a lower percentage ($p < 0.0000$) of atopy than workers in other industries, such as isocyanate manufacturing, where non-IgE mechanisms likely predominate (Table 5). This may indicate "self-selection" among the atopic employees, who leave the workplace because of respiratory problems (48).

In vitro experiments are useful correlates of epidemiologic studies. Heterogeneous mixtures, such as cotton dust extracts, are ideally suited to analyses such as crossed immunoelectrophoresis (CIE). CIE and modifications of this technique, including crossed radioimmunoelectrophoresis (CRIE), are used for the separation and identification of antigens or allergens, respectively. Briefly, CIE is a two-dimensional electrophoretic technique that employs hyperimmune rabbit antisera raised against a crude antigenic extract to obtain a number of precipitin arcs. Results of CIE using ACDE and hyperimmune rabbit anti-ACDE antisera showed that ACDE is immunogenic in the rabbit. As seen in a photograph of the stained plate (Fig. 3a) and a drawing (Fig. 3b)

with the precipitins arbitrarily numbered, 51 separate antigenic components were detected. No precipitins were detected in pre-immunization sera (29).

CRIE demonstrated that IgE-specific antibodies were present in 4/11 oil mill workers' sera tested (29). Peaks 16, 17, 22, 43, and 51 were allergenic in man (Fig. 4). All of the individuals had a RAST value strongly positive to cotton linter extract. Nonspecific radiostaining in sera from seven marginal RAST positives was not seen.

Immunoelectrophoretic techniques can also be used to identify specific antigenic/allergenic components. We have shown, using rocket immunoelectrophoresis (37), that antibodies directed against fungal components are present in the rabbit anti-cotton antisera. We recently extended the study of anti-fungal antibodies in "cotton anti-sera" using the CIE technique. Fungal extracts separated by electrophoresis in the first dimension were then electrophoresed into anti-cotton antisera for the second dimension. Thirteen precipitin arcs were demonstrated with the hyperimmune anti-ACDE sera when *Alternaria tenuis* was used as the extract (Fig. 5). Results of experiments with fungal extracts are presented in Table 6. The finding of fungal contamination of cotton dust is not

Table 6. Results of crossed immunoelectrophoresis (CIE) with fungal extracts against hyperimmune rabbit anti-ACDE antisera.

Fungal species	Number of lines detected on CIE	
	Pre-immunization	Post-immunization
<i>Alternaria tenuis</i>	1	13
<i>Aspergillus niger</i>	1	2
<i>Fusarium solani</i>	0	4
<i>Hormodendrum hordei</i>	0	0
<i>Penicillium notatum</i>	0	0

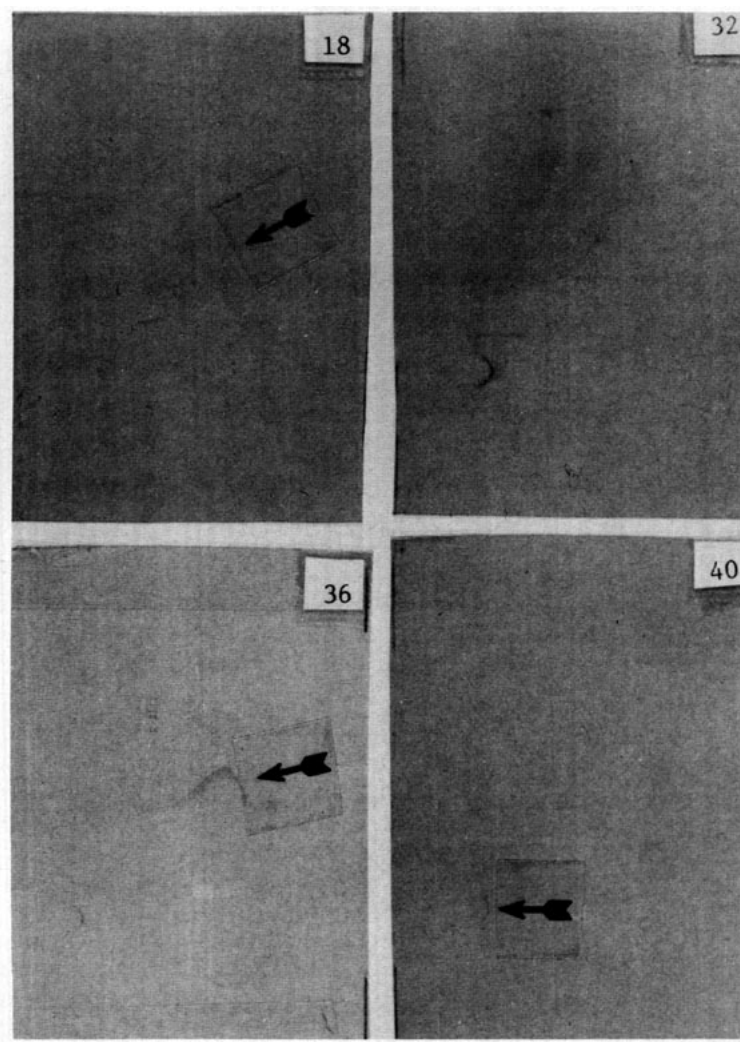


FIGURE 4. Crossed radioimmuno-electrophoresis (CRIE) pattern of 4 oil mill worker's sera and aqueous cotton dust extract (ACDE). From O'Neil et al. (29). Reproduced with permission of S. Karger AG, Basel.

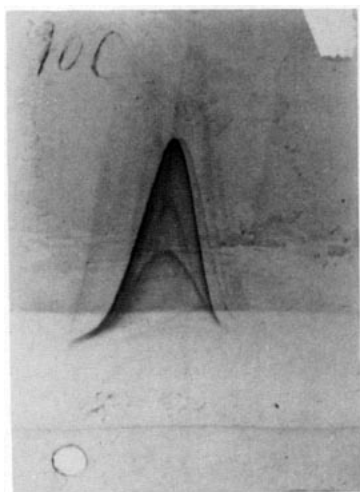


FIGURE 5. Crossed immunoelectrophoresis (CIE) pattern of *Alternaria tenuis* extract against hyperimmune rabbit anti-ACDE antisera. From O'Neil et al. (29). Reproduced with permission of S. Karger AG, Basel.

new, but our work represents the first demonstration of the antigenic nature of the fungal material in ACDE (29).

While these findings are strongly indicative of an IgE-mediated hypersensitivity to cotton dust component(s) in the pathogenesis of classic byssinosis, this remains to be confirmed. We have demonstrated that aqueous extracts of cotton dust are, however, immunogenic in the rabbit and allergenic in man, and a significant portion of the immune response is directed against fungal contaminants of the dust. Further, our epidemiologic studies suggest that an immunologic dysfunction, such as atopy, may be a risk factor in the development of cotton dust induced respiratory disease.

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